Reproductive Effects in Birds Exposed to Pesticides and Industrial Chemicals

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Environmental contamination by agricultural chemicals and industrial waste disposal results in adverse effects on reproduction of exposed birds. The diversity of pollutants results in physiological effects at several levels, including direct effects on breeding adults as well as developmental effects on embryos. The effects on embryos include mortality or reduced hatchability, failure of chicks to thrive (wasting syndrome), and teratological effects producing skeletal abnormalities and impaired differentiation of the reproductive and nervous systems through mechanisms of hormonal mimicking of estrogens. The range of chemical effects on adult birds covers acute mortality, sublethal stress, reduced fertility, suppression of egg formation, eggshell thinning, and impaired incubation and chick rearing behaviors. The types of pollutants shown to cause reproductive effects include organochlorine pesticides and industrial pollutants, organophosphate pesticides, petroleum hydrocarbons, heavy metals, and in a fewer number of reports, herbicides, and fungicides. *o,p'*-DDT, polychlorinated biphenyls (PCBs), and mixtures of organochlorines have been identified as environmental estrogens affecting populations of gulls breeding in polluted "hot spots" in southern California, the Great Lakes, and Puget Sound. Estrogenic organochlorines represent an important class of toxicants to birds because differentiation of the avian reproductive system is estrogen dependent. — Environ Health Perspect 103(Suppl 7):165–171 (1995)

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Introduction

Wild birds are very conspicuous in the landscape. Injuries to populations of birds from environmental pollutants and pesticides are obvious indicators of environmental damage. Rachel Carson's *Silent Spring* (*1*) identified the urban use of pesticides (primarily DDT) as the cause of a noticeable decline of birds singing in the eastern United States and also the cause of mass songbird mortalities. While direct exposure to DDT is not highly toxic to birds, heavy and repetitive use of the pesticide is. DDT was used aggressively to kill the beetles that spread Dutch Elm disease; this resulted in the bioaccumulation of DDE in nontarget species of earthworms. The levels were high enough that robins and other songbirds which ingested the earthworms received lethal doses of the pesticide, resulting in large losses of urban birds.

Contemporaneous with DDT use to control Dutch elm disease, DDD (a DDT analog) was used to control gnats in Clear Lake, California (*2,3*); this resulted in the first well-documented ecological magnification of an organochlorine in invertebrates, fish, and birds. Nesting western grebes exhibited breeding failure after the first applications of DDD to the lake in 1949 and significant adult mortality after treatment in 1954. Eggshell thinning and embryo mortality were evident in nesting grebes through 1971, 14 years after the last DDD treatment.

Silent Spring emphasized mortality of birds due to biomagnification of organochlorines and eggshell thinning by DDT metabolites as primary causes of reproductive impairment. Subsequent research has also identified other pesticides and industrial chemicals that cause mortality and reproductive impairment, which affects both embryos and adult birds. The effects on embryos include mortality or reduced hatchability, failure of chicks to thrive (wasting syndrome), and teratological effects that produce skeletal abnormalities and impaired differentiation of the reproductive and nervous systems through mechanisms of hormonal mimicking of estrogens. The range of chemical effects on adult birds covers acute mortality, sublethal stress, reduced fertility, suppression of egg formation, eggshell thinning, and impaired incubation and chick rearing behaviors (*4*). The types of pollutants shown to cause reproductive effects include organochlorine pesticides and industrial pollutants, organophosphate pesticides, petroleum hydrocarbons, heavy metals, and in a fewer number of reports, herbicides, and fungicides.

Background Studies

The urban and agricultural use of DDT and other organochlorine pesticides resulted in localized high-residue levels in soils and plants; runoff resulted in significant aquatic residues in estuaries, resulting in bioaccumulation by fish and predatory birds. For example, reproductive failure of grebes (*3*), ospreys (*5*), peregrine falcons (*6,7*), and bald eagles (*8,9*) was caused primarily by urban and agricultural insect control measures. Point source pollution from manufacturing plants was responsible for reproductive failure of cormorants and pelicans in southern California (*10–13*). Breeding failures and high organochlorine levels in aquatic birds nesting in the Great Lakes implicated DDE, polychlorinated biphenyls (PCBs), dioxins, and dibenzofurans (*14–16*).

Eggshell thinning and breeding failure of raptors and seabirds (*11,14,17,18*) were documented with DDT and its metabolites (*19*). DDT, however, was not the only pesticide or pollutant to affect reproduction in birds. Other persistent organochlorine pesticides with documented effects

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Abbreviations used: DDT, dichlorodiphenyltrichloroethane; DDD, dichlorodiphenyldichloroethane; PCBs, polychlorinated biphenyls; HCH, hexachlorocyclohexane; HCB, hexachlorobenzene; PGC, primordial germ cells; AFP, α-fetoprotein; DES, diethylstilbestrol; GLEMEDS, Great Lakes embryo mortality, edema, and deformity syndrome; PAHs, polyaromatic hydrocarbons.

included dieldrin, endrin, aldrin, mirex, kepone, chlordane, toxaphene, hexachlorobenzene, and lindane (*20*). Most of the organochlorines contributed only in a minor way compared to DDT and dieldrin, and most were banned from use in the United States in the early 1970s. Many continue to be used in Asia, Africa, and South America (*21,22*).

In estuaries, the environmental buildup of industrial chemicals such as PCBs and dioxins also occurred during the 1950s and 1960s. Documented exposures to birds, especially in the Great Lakes (*23*), Long Island Sound (*24*), Puget Sound (*25,26*), and San Francisco and San Diego Bays (*27,28*), were correlated with declines in populations of fish-eating birds. In these cases, the causative agent(s) was difficult to pinpoint because several organochlorines and heavy metals (copper, mercury, selenium, tributyl tin) were often found in complex mixtures.

Organochlorine Compounds

Organochlorine compounds include PCBs, dioxins, dibenzofurans, and many persistent pesticides including DDT, kepone, cyclodiene pesticides (dieldrin, aldrin, and endrin), lindane (HCH), and hexachlorobiphenyl (HCB), toxaphene, and chlordane. Most of the persistent organochlorine pesticides have been removed from the market in Europe and North America, but many are still in use in Africa, Asia, and South America. Endosulfan, methoxychlor, and dicofol remain in use in the United States because their persistence and potential for bioaccumulation is lower. PCBs, dibenzodioxins, and dibenzofurans became environmental pollutants only through industrial waste disposal, waste incineration, or as contaminants in the herbicides 2,4-D and 2,4,5-T. PCB disposal is now largely a historical problem rather than a continuing input into the environment. Incinerators and kraft-process paper pulp bleaching mills are still sources of dibenzodioxins and dibenzofurans, but the discharge of dioxins is being reduced by conversion of pulp mills to nonchlorine bleaching processes (*29*).

Most classes of organochlorines are represented by many congeners (75 dibenzodioxins, 135 dibenzofurans, and 209 PCBs), and complex mixtures of these organochlorines are typically found together in environmental samples (sediments, tissue, or egg residues). The acute toxicity of individual congeners varies over several orders of magnitude, with the 2,3,7,8-chlorinated dioxins and coplanar PCBs being the most toxic (*30,31*).

At Scotch Bonnet Island in Lake Ontario and on Santa Barbara Island in southern California, observed abnormal behaviors of breeding gulls were associated with organochlorine pollutants (*32,33*), leading to the hypothesis that *o,p'*-DDT, mirex, and some PCBs are estrogenic at environmental concentrations and responsible for hormonal disruptions of breeding birds and abnormalities in their offspring (*34,35*).

Field and laboratory data gathered over the past two decades indicated that the mechanisms of action of organochlorine pollutants on reproduction in birds are quite diverse. The field observations and the effects seen in birds have been very useful in predicting effects in other wildlife species, such as mink, snapping turtles, or alligators (*36–38*). Hormone-disrupting effects are a result of organochlorines mimicking the action of estrogens; the xenobiotic hormone disruptions are complex, and effects in birds and mammals may be quite dissimilar because steroid hormone control of reproductive system differentiation by estrogens and androgens is different in mammals and birds. An understanding of the control of differentiation of the reproductive tracts in mammals and birds is necessary to understand differences in xenobiotic estrogen effects in these two classes of vertebrates.

Sexual Differentiation of Birds and Mammals

In both birds and mammals, sex determination and control of differentiation are linked to the heterogametic sex. In mammals the heterogametic sex is the male, with a XY sex chromosome complement (female XX), while in birds the female is the heterogametic sex (ZW, with male being ZZ). In birds and mammals, the homogametic sex is the "default" sex, i.e., the phenotype into which the embryo will develop in the absence of the sex-specific hormones and other compounds that cause sex differentiation. In mammals the default sex is female, and without expression of specific gene products initiated by genes on the Y chromosome, the embryo will differentiate into a phenotypic female. In birds, the default sex is male (ZZ); phenotypic differentiation of the embryo into a male will occur unless specific female gene products are translated and estradiol is synthesized, causing differentiation of the gonad into an ovary.

In both birds and mammals, the initial organization of the gonad is similar, with an indifferent gonad developing as a genital ridge protruding into the dorsal body cavity cranial to the kidneys. In mammals, expression of the testicular organizer gene located on the Y chromosome induces condensation of cells into the presumptive seminiferous tubules (*39*). Primordial germ cells (PGC), which originate as extraembryonic cells in the yolk sac of both birds and mammals, migrate into the indifferent gonads at mid-gestation. In mammals, the PGC become localized in the default location of the ovarian cortex or, under the influence of testosterone, the PGC migrate into the seminiferous tubules and become the primary spermatogonia.

In birds, the PGC migrate into the default location of the seminiferous tubules in males (*40*). Development of the ovarian architecture occurs when estradiol is synthesized by the gonad, causing localization of the PGC in the cortex of the left ovary rather than in the presumptive seminiferous tubules. Estradiol additionally causes regression of the right gonad and suppression of the synthesis of a glycoprotein, Mullerian regression factor, which, in the absence of estrogen, causes regression of the developing oviducts in males. Estradiol is responsible for retention of the left Mullerian duct and for its differentiation into the functional left oviduct and shell gland.

Because of the differences in steroid hormonal control of sexual differentiation in birds and mammals, xenobiotic estrogens have different effects on birds and mammals during embryonic development. In male birds, the primary differentiation of the testes will be altered by estrogens that stimulate PGC to become localized in the cortex of the gonad in addition to the normal development of seminiferous tubules and localization of the normal PGC within the medulla of the gonad. The number of PGC that become localized in the cortex is estrogen dose dependent, with low doses resulting in only a few PGC localized in the cortex and large doses resulting in a layer of cortical PGC similar to the cortex of an ovary (*35*). The cortical PGC differentiate into primordial follicles by entering into meiotic prophase, typical of ovarian PGC. The seminiferous tubules are retained with a reduced number of PGC in the gonadal medulla of estrogen-treated males; they provide histological evidence of the male genetic sex of the embryo, even in embryos exposed to high levels of estradiol in which the gross shape

of the gonad is converted to the ovarian form. Estrogen exposure to female embryos does not cause alteration of the structure of the ovary in birds, but does result in oviduct changes.

Differentiation of the accessory sexual ducts is altered by estrogen exposure in a dose-dependent fashion. In male gulls and in chickens, the left oviduct may be retained in males at hatching, in addition to the retention of the vasa deferentia (*40,41*). The right oviduct is frequently retained as a small edematous vestigial tubule, not usually more than 5 to 6 mm in length. In females, the left oviduct is not grossly altered, but an abnormally large right oviduct may be retained. Functional changes in the left oviduct and shell gland may occur when female chicks reach adulthood, as exposed birds lay eggs with defective, thin, or soft shells (*42*).

In mammals, the effect of exogenous estrogen exposure is qualitatively different than in birds because testosterone controls the differentiation of the gonads. Estrogen is normally present in the fetal circulation from maternal sources but is sequestered by α-fetoprotein (AFP), which has a high estrogen binding affinity and protects the fetal tissues from estrogen exposure (*39*). It would be expected, therefore, that androgen analogs would have much more of an effect on primary sexual differentiation in mammals, in contrast to the estrogen effects in birds.

In mammals, the dose dependency and effects of exogenous estrogens may be species specific because of the large species variation in circulating maternal estrogens and circulating levels of AFP, which bind estrogens, reducing or preventing estrogen binding to fetal tissue receptors. Xenobiotic estrogens are made up of many chemical forms, however, and may not bind with equal affinity to the estrogen receptor and AFP, possibly resulting in circulating levels of xenobiotic estrogens that exceed the binding capacity of AFP in the fetal circulation. Estrogens do not control differentiation of the primary sexual apparatus in mammals, but they do have modifying effects on the uterus and brain, as has been demonstrated with human fetal exposure to DES (*43*).

Reproductive Effects of Xenobiotic Estrogens on Bird Embryos

To date the only xenobiotic estrogens that have been identified to have effects on avian differentiation have been lipophilic organochlorines, which bioaccumulate and are deposited into the yolk of eggs. Several organochlorine compounds have been identified as weak estrogens or precursors of estrogens produced by liver hydroxylation (*44–46*). Organochlorine pesticides identified as estrogenic include kepone, *o,p'*-DDT, methoxychlor, endosulfan, and dicofol (*34,46–48*).

o,p'-DDT or methoxychlor injection into gull eggs mimics the action of estrogens or DES (*41*) and results in abnormalities of both male and female embryos. Males are feminized, with primordial germ cells becoming localized in the cortex of the gonad as well as in the seminiferous tubules. In gulls, the amount of ovarian-type cortical tissue is dose dependent, as is the retention of the left and right oviduct (*35*). In females, the right oviduct may be only partially regressed. The qualitative effects of *o,p'*-DDT or methoxychlor were similar to estradiol treatment, with estradiol being more than 100-fold more potent than *o,p'*-DDT.

Estrogen receptor binding studies with *o,p'*-DDT, methoxychlor, or their hydroxylated metabolites (*47*) indicate that the binding affinity of the parent pesticide is low, but a hydroxylated metabolite may have a binding affinity very similar to estradiol. Significant hormonal activity can be produced when only a fraction of the parent pesticide is metabolized. The extent of organochlorine pollutant metabolism is dependent upon liver enzyme induction, and liver mixed-function oxidases become inducible by several classes of organochlorines midway through embryonic development (*49*). Avian embryos are particularly at risk from metabolite activation because the metabolite products are not excreted from the egg but remain in the blood circulation throughout incubation. The normal nitrogenous metabolic wastes are sequestered in the allantois as a semisolid slurry of urates, and water-soluble metabolites of xenobiotics will remain in the circulation.

Hydroxylated PCB congeners also have been identified as estrogenic (*50,51*), with a large variation in potency between hydroxylated metabolites. In experimental studies the enzymatic hydroxylation can be manipulated by the dosing regimen, with small initial doses of PCBs causing the induction of liver microsomal enzymes and subsequent larger doses being rapidly hydroxylated to active estrogens (*52*). Most of the hydroxylated metabolites formed are more water soluble than the parent congeners, and most were thought to be excreted in the urine of mammals (*53*). However, retention of 13 hydroxylated PCB metabolites at high levels has recently been shown in humans, rats, and seals (*54*), indicating both that hydroxylation is a widespread metabolic pathway and that hydroxylated metabolites may be retained in the circulation or fat.

Other Classes of Xenobiotic Estrogens

Alkyl phenols, widely used as wetting agents, surfactants, and industrial chemical additives, are also estrogenic (*55*) and have been demonstrated to have adverse effects on fish downstream of municipal wastewater discharges. In response to alkyl phenol exposure, male fish are reported to synthesize vitellogenin, an estrogenic protein synthesis by the liver and normally expressed only in females. No studies to date have implicated alkyl phenols as being estrogenic in birds.

Many plants synthesize isoflavanoid phytoestrogens, which may have either estrogenic agonist or antiestrogenic effects on estrogen receptor binding (*56*). Most do not have significant effects on avian reproduction but may be used as chemical cues to modify reproduction. Variations in levels of isoflavanoids in clover and other legumes have been implicated in affecting reproduction in wild quail (*57*).

The extent to which animals are at risk from estrogenic xenobiotics is difficult to estimate. Animals have been exposed to phytoestrogens for many generations and have apparently developed metabolic pathways to adjust to this exposure from natural sources. Most hydroxylated metabolites of organochlorines are weak estrogens, they are unlikely to be bioaccumulated because of their water solubility, and they will be excreted in the urine of adult animals before reaching concentrations high enough to adversely affect adult reproductive function. Embryonic animals, however, are sensitive to permanent developmental effects of estrogens, and the risk of exposure to hydroxylated organochlorines is largely unknown. The binding constants for organochlorines to AFP may be very different from binding constants for steroidal estrogens, and exposure of the fetus will be a complex function of maternal or fetal liver hydroxylation, estrogen receptor binding, AFP sequestration, and maternal urinary excretion. The exposure risk for avian embryos is also very different from that of mammals. The embryonic liver is capable of responding to induction

by organochlorines, as mixed-function oxidases are active in the last half of incubation (*49*). If hydroxylated metabolites with estrogenic activity are produced, they will remain in the embryonic circulation throughout embryonic development because water is recycled within the egg as nitrogenous wastes are sequestered as semisolid urates in the allantois. Avian embryos are also at higher risk than mammalian embryos because the phenotypic determination of sex is estrogen dependent. The opportunity for embryonic exposure to much higher concentrations of xenobiotic estrogens is also a factor because the lipophilic organochlorines are selectively deposited in the high lipid yolk of eggs, especially in raptors and fish-eating birds. The combination of food chain exposure and estrogen dependence of sexual differentiation is almost certainly responsible for the field observations of estrogenic effects in birds. To date, only colonial fish-eating birds breeding in "hot spots" of contamination have been reported with estrogenic developmental defects.

The likelihood that gulls in the wild were suffering population effects of estrogenization of males was tested by Fry and coworkers (*34,35*); experiments demonstrated that levels of DDT found in eggs in southern California caused feminization of male embryos. They postulated that the temporal and geographical localization of female–female pairing, supernormal clutches of eggs in gull nests, and sex ratio skew of breeding gulls was due to reduced numbers of male breeders at the colony. These effects were due to feminization of embryos and either chemical neutering of the hatched chicks or differential survival of chicks.

In this review, only direct effects of pollutants on the reproductive system have been examined. Developmental effects on behavior also occur with *in ovo* exposure of estrogens, which have the potential to alter the structure and function of the central nervous system. The complex topic of estrogen control of brain differentiation and behavior has been recently addressed by Adkins-Regan et al. (*58*).

Other Effects of Pollutants on Avian Embryos and Chicks

Egg-borne pollutants and direct application of pollutants to eggs have been documented to cause mortality, reduced hatchability, terata, and reduced survival of chicks hatched from eggs. In addition to organochlorines as causative agents, heavy metals, petroleum hydrocarbons, and pesticides have been identified. Adverse effects on survival and hatchability of eggs are documented with bioaccumulation of dioxins (*59,60*) and selenium (*61*), both of which are pollutants more toxic to embryos than to adults and which bioaccumulate into yolk to concentrations that become toxic to embryos. Sublethal effects produced by these compounds include subcutaneous and cardiac edema and terata of the beak, axial skeleton, and heart. Several sites within the Great Lakes that are polluted with PCBs and other organochlorines have produced abnormalities in embryos and chicks of bald eagles, cormorants, gulls, and terns. The consistent pattern of edema, beak malformations, cardiac edema, and skeletal malformations has been termed GLEMEDS (Great Lakes embryo mortality, edema, and deformity syndrome); the syndrome correlates with dioxin toxic equivalents, which are primarily a result of bioaccumulation of coplanar PCB congeners (PCB 126, 169, and 77 being the most toxic congeners). Selenium contamination through bioaccumulation from food-chain magnification of agricultural drainwater metals in high selenium soils in several low rainfall regions has been responsible for deformities of waterfowl and shorebirds in the Kesterson Wildlife Refuge, California (*61*), and in Nevada (*62*).

Direct application of toxicants at high concentrations to wild bird eggs probably occurs rarely in the wild, but it may occur through transfer of contaminants such as spilled petroleum oil from the plumage of contaminated incubating birds or from direct application of agricultural chemicals to eggs in nests adjacent to agriculture. Hoffman (*63*) has reviewed this data and summarized the toxicity and teratogenicity of products applied directly to eggs. Mortality and reduced hatchability of eggs were caused by petroleum oils [the most toxic components are the polyaromatic hydrocarbons (PAHs)], organophosphate insecticides, some herbicides (paraquat, trifluralin, prometon, and others) and fungicides (maneb). Dose dependency of many of the products has not been established for direct application toxicity because the solvent vehicle is an important determinant of toxicant penetration through the eggshell and, therefore, for exposure determination. Most of the products tested were also teratogenic at sublethal levels. The PAH fractions of petroleum oils produced liver necrosis, edema of heart, and enlargement of the spleen (*64–66*). Refined oils and crankcase oils studied by several workers reviewed by Hoffman (*63*) were also toxic and teratogenic, with PAHs and metal contaminants being most toxic. Organophosphate insecticides caused axial skeleton malformations (scoliosis and lordosis), edema, and stunted growth. The toxicity was compared with application rates, and relative risk for the organophosphates tested was highest for malathion, with decreasing risk for dimethoate, diazinon, parathion, and acephate. Carbamate insecticides did not cause terata at expected environmental exposure concentrations, and of nine fungicides tested only maneb caused malformations (*63*).

Reproductive Effects in Adult Birds

Mortality of birds is not a specific reproductive effect, but on a population level, reproduction is impaired due to decreased numbers of breeding birds and decreased fitness of remaining adults. Sublethal exposures may adversely affect reproduction through nonspecific morbidity or increased stress, which results in cessation of lay, interruption of incubation, or reduced care of chicks. Petroleum oil exposure to breeding birds, either by exposure of the plumage or by ingestion of oil, causes increased stress with elevated circulating corticosterone and apparent feedback down regulation of reproduction at the pituitary level (*67–69*). Oil exposure will cause cessation of egg yolk formation (*70*), which results in reduced lay or abandonment of breeding. Laboratory exposure studies have been reviewed by Albers (*71,72*), demonstrating considerable variability in sensitivity to hydrocarbon induced hemolytic anemia (*73–75*) and induction of liver mixed-function oxidases (*76*)—effects that appear to contribute to increased stress and reduced breeding success. Field studies (*77*) have shown that exposure to 0.1- to 2.0-ml weathered crude oil is sufficient to prevent or impair egg formation and egg laying, incubation, and stability of the pair bond. Trivelpiece et al. (*69*) showed that field exposure of adult storm-petrels during the chick-rearing period caused reduced foraging and food delivery by adults, which resulted in decreased growth or death of chicks.

The best documented and notorious effect of environmental pollutants on birds is eggshell thinning caused by DDE; this results in crushed eggs and breeding failure of many sensitive raptorial and fish-eating

birds (*10,17,18*). Eggshell thinning is correlated with DDE inhibition of shell gland calcium ATPase (*78,79*), and the species most susceptible to eggshell thinning appear to have reduced ability to metabolize organochlorines (*80*). Whether the increased sensitivity to eggshell thinning is related to differences in liver metabolism of organochlorines is unknown.

Organochlorine pollutants and organophosphate pesticides may also influence the breeding bahavior of exposed birds. Herring gulls breeding on Scotch Bonnet Island, Lake Ontario, showed decreased incubation attentiveness and decreased defense of territories correlated with a mix of organochlorines (*32,81*). Incubation and chick-rearing behavior impairment has also been correlated with organochlorine exposure to ring doves (*82*) and merlins (*83*). Parathion has been correlated with altered incubation behavior in experimentally exposed mallards and laughing gulls (*84,85*).

The effects of pollutants on reproduction are mediated at many different physiological levels. The diversity and extent of effects have been impossible to predict because many of the biochemical mechanisms of the side effects of agricultural chemicals are unrelated to the specific mechanisms of action of the designed use. The unexpected side effects, such as eggshell thinning by DDE or estrogenic effects of *o,p'*-DDT, could not be predicted before initial use of the compounds, and the bioaccumulation and ecological magnification consequences were not anticipated. The positive value of monitoring wild bird populations has been demonstrated with the observations of ecological injury that have occurred with misuse of pesticides and irresponsible pollutant disposal. The adverse effects of chemicals on wildlife have been signals for revision of laws and implementation of new regulations to prevent adverse effects on human populations, but only through continued wildlife monitoring will new, unexpected side effects of chemicals in the environment be observed and corrected.

REFERENCES

1. Carson R. Silent Spring. Boston:Houghton Mifflin, 1962.
2. Rudd RL. Pesticides and the Living Landscape. Madison, WI:University of Wisconsin Press, 1964.
3. Rudd RL, Herman SG. Toxic effect of pesticide residues on wildlife. In: Environmental Toxicology of Pesticides (Matsumura F, ed). New York:Academic Press, 1972;471–485.
4. Gilman AP, Peakall DB, Hallett DJ, Fox GA, Norstrom RJ. Herring gulls (*Larus argentatus*) as monitors of contamination in the Great Lakes. Animals as Monitors of Environmental Pollution. Washington:National Academy of Sciences, 1977;280–289.
5. Spitzer PR, Risebrough RW, Walker W 2d, Hernandez R, Poole A, Puleston D, Nisbet IC. Productivity of ospreys in Connecticut-Long Island increases as DDE residues decline. Science 202:333–335 (1978).
6. Cade TJ, Lincer JL, White CM, Roseneau DG, Swartz LG. DDE residues and eggshell changes in Alaskan falcons and hawks. Science 172:955–957 (1971).
7. Peakall DB. DDE: its presence in peregrine eggs in 1948. Science 183:673–674 (1974).
8. Cromartie E, Reichel WL, Locke LN, Belisle AA, Kaiser TE, Lamont TG, Mulhern BM, Prouty RM, Swineford DM. Residues of organochlorine pesticides and polychlorinated biphenyls and autopsy data for bald eagles, 1971–1972. Pestic Monit J 9:11–14 (1975).
9. Grier JW. Ban of DDT and subsequent recovery of reproduction in bald eagles. Science 218:1232–1235 (1982).
10. Gress F, Risebrough RW, Anderson DW, Kiff LF, Jehl JR. Reproductive failures of double-crested cormorants in southern California and Baja California. Wilson Bull 85:197–208 (1973).
11. Anderson DW, Hickey JJ. Oological data on egg and breeding characteristic of brown pelicans. Wilson Bull 82:14–28 (1970).
12. Anderson DW, Jehl JR, Risebrough RW, Woods LA, Deweese LR, Edgecomb WG. Brown pelicans: improved reproduction off the southern California coast. Science 190:806–808 (1975).
13. Blus LJ, Thair GL, Burkett SN Jr . Effects of organochlorine residues on eggshell thickness, reproduction, and population status of brown pelicans (*Pelecanus occidentalis*) in South Carolina and Florida, 1969-76. Pestic Monit J 12:172–184 (1979).
14. Keith JA. Reproduction in a population of herring gulls (*Larus argentatus*) contaminated by DDT. J Applied Ecol 3(Suppl):57–70 (1966).
15. Harris HJ, Erdman TC, Ankley GT, Lodge KB. Measures of reproductive success and polychlorinated biphenyl residues in eggs and chicks of Forster's terns on Green Bay, Lake Michigan, Wisconsin—1988. Arch Environ Contam Toxicol 25:304–314 (1993).
16. Gilbertson M, Fox GA. Pollutant-associated embryonic mortality of Great Lakes herring gulls. Environ Pollut 12:211–216 (1977).
17. Hickey JJ, Anderson DW. Chlorinated hydrocarbons and eggshell changes in raptorial and fish-eating birds. Science 162:271–273 (1968).
18. Cooke AS. Shell thinning in avian eggs by environmental pollutants. Environ Pollut 4:85–152 (1973).
19. Abou-Donia MB, Menzel DB. The metabolism *in vivo* of 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT), 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane (DDD) and 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene (DDE) in the chick by embryonic injection and dietary ingestion. Biochem Pharmacol 17:2143–2161 (1968).
20. Nisbet ICT. Ecological magnification. Technol Rev 3–4:6–8 (1975).
21. Boardman R. Pesticides in World Agriculture. London:Macmillan, 1986.
22. Asian Development Bank. Handbook on the Use of Pesticides in the Asia-Pacific Region. Manila, Philippines:Asian Development Bank, 1987.
23. Peakall DB. Known effects of pollutants on fish-eating birds in the Great Lakes of North America. In: Toxic Contamination in Large Lakes. Vol I (Schmidtke NW, ed). Chelsea, MI:Lewis Publishers, 1988;39–54.
24. Hays H, Risebrough RW. Pollutant concentrations in abnormal young terns from Long Island Sound. Auk 89:19–35 (1972).
25. Henny CJ, Blus LJ, Grove RA. Western grebe, *Aechmophorus occidentalis*, wintering biology and contaminant accumulation in Commencement Bay, Puget Sound, Washington. Can Field Nat 104:460–472 (1990).
26. Malins DC, McCain BB, Brown DW, Sparks AK, Hodgins HO. Chemical contaminants and biological abnormalities in central and southern Puget Sound. NOAA Technical Memorandum OMPA-2. Boulder, CO:National Oceanic and Atmospheric Administration, 1980.
27. Ohlendorf HM, Custer TW, Lowe RW, Rigney M, Cromartie E. Organochlorines and mercury in eggs of coastal terns and herons in California, U.S.A. Colon Waterbirds 11:85–94 (1988).

28. Ohlendorf HM, Marois KC. Trace elements and organochlorines in surf scoters from San Francisco Bay, 1985. Environ Monit Assess 18:105–122 (1991).
29. Colodey AG, Wells PG. Effects of pulp and paper mill effluents on estuarine and marine ecosystems in Canada: a review. J Aquat Ecosystem Health 1:201–226 (1992).
30. Ankley GT, Tillitt DE, Giesy JP, Jones PD, Verbrugge DA. Bioassay-derived 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents in PCB-containing extracts from the flesh and eggs of Lake Michigan chinook salmon (*Oncorhynchus tshawytscha*) and possible implications for reproduction. Can J Fish Aquat Sci 48:1685–1690 (1991).
31. Tanabe SN, Kannan N, Subramanian AN, Watanabe S, Tatsukawa R. Highly toxic coplanar PCBs: occurrence, source, persistency and toxic implications to wildlife and humans. Environ Pollut 47:147–163 (1987).
32. Fox GA, Gilman AP, Peakall DB, Anderka FW. Behavioral abnormalities of nesting Lake Ontario herring gulls. J Wildl Manage 42:477–483 (1978).
33. Hunt GL, Hunt MW. Female-female pairing in western gulls in southern California. Science 196:1466–1467 (1977).
34. Fry DM, Toone CK. DDT-induced feminization of gull embryos. Science 213:922–924 (1981).
35. Fry DM, Toone CK, Speich SM, Peard RJ. Sex ratio skew and breeding patterns of gulls demographic and toxicological considerations. Stud Avian Biol 10:26–43 (1987).
36. Heinz GH, Percival HF, Jennings ML. Contaminants in American alligator eggs from Lake Apopka, Lake Griffin, and Lake Okeechobee, Florida. Environ Monit Assess 16:277–28 (1991).
37. Bishop CA, Brooks RJ, Carey JH, Ng P. The case for a cause-effect linkage between environmental contamination and development in eggs of the common snapping turtle (*Chelydra serpentina*) from Ontario, Canada. J Toxicol Environ Health 33:521–547 (1991).
38. Woodward AR, Percival HF, Jennings ML, Moore CT. Low clutch viability of American alligators on Lake Apopka. Fla Sci 56:52–63 (1993).
39. vom Saal FS, Montano MM, Wang MH. Sexual differentiation in mammals. In: Advances in Modern Environmental Toxicology. Vol XXI (Mehlman MA, ed). Princeton, NJ:Princeton Scientific Publishing, 1992;17–84.
40. Romanoff AJ. The Avian Embryo. New York:Macmillan, 1960.
41. Boss WR, Witschi E. The permanent effects of early stilbestrol injections on the sex organs of the herring gull (*Larus argentatus*). J Exp Zool 105:61–77 (1947).
42. Greenwood AW, Blyth JSS. Experimental modification of the accessory sexual apparatus in the hen. Q J Exp Physiol 28:61–69 (1938).
43. Hines M. Surrounded by estrogens? Considerations for neurobehavioral development in human beings. In: Advances in Modern Environmental Toxicology. Vol XXI (Mehlman MA, ed). Princeton, NJ:Princeton Scientific Publishing, 1992;261–282.
44. Kupfer D, Bulger WH. Metabolic activation of pesticides with proestrogenic activity. FASEB J 46:1864–1889 (1987).
45. Eroschenko VP. Estrogenic activity of the insecticide chlordecone in the reproductive tract of birds and mammals. J Toxicol Environ Health 8:731–742 (1981).
46. Eroschenko VP, Palmiter RD. Estrogenicity of kepone in birds and mammals. In: Estrogens in the Environment (McLachlan JA, ed). New York:Elsevier, 1980;305–325.
47. Bulger WH, Muccitelli RM, Kupfer D. Studies on the *in vivo* and *in vitro* estrogenic activities of methoxychlor and its metabolites. Role of hepatic mono-oxygenase in methoxychlor activation. Biochem Pharmacol 27:2417–2423 (1978).
48. Soto AM, Lin T-M, Justicia H, Silvia RM, Sonnenschein C. An "in culture" bioassay to assess the estrogenicity of xenobiotics (e-screen). In: Advances in Modern Environmental Toxicology. Vol XXI (Mehlman MA, ed). Princeton, NJ:Princeton Scientific Publishing, 1992;295–310.
49. Rodman LE, Shedlofsky SI, Mannschreck A, Puttmann M, Swim AT, Robertson LW. Differential potency of atropisomers of polychlorinated biphenyls on cytochrome P450 induction and uroporphyrin accumulation in the chick embryo hepatocyte culture. Biochem Pharmacol 41:915–922 (1991).
50. Korach KS, Sarver P, Chae K, McLachlan JA, McKinney JD. Estrogen receptor-binding activity of polychlorinated hydroxybiphenyls: conformationally restricted structural probes. Mol Pharmacol 33:120–126 (1988).
51. Soontornchat S, Li MH, Cooke PS, Hansen LG. Toxicokinetic and toxicodynamic influences on endocrine disruption by polychlorinated biphenyls. Environ Health Perspect 102:568–571 (1994).
52. Li MH, Zhao YD, Hansen LG. Multiple dose toxicokinetic influence on the estrogenicity of 2,2′,4,4′,5,5′-hexachlorobiphenyl. Bull Environ Contam Toxicol 53:583–590 (1994).
53. Sparling J, Fung D, Safe S. Bromo- and chlorobiphenyl metabolism: GC/MS identification of urinary metabolites and the effects of structure on their rates of excretion. Biomed Mass Spectrom 7:13–20 (1980).
54. Bergman A, Klasson-Wehler E, Kuroki H. Selective retention of hydroxylated PCB metabolites in blood. Environ Health Perspect 102:464–469 (1994).
55. White R, Jobling S, Hoare SA, Sumpter JP, Parker MG. Environmentally persistent alkylphenolic compounds are estrogenic. Endocrinology 135:175–182 (1994).
56. McLachlan JA, Newbold RR, Korach KS, Hogan M. Risk assessment considerations for reproductive and developmental toxicity of œstrogenic xenobiotics. In: Human Risk Assessment: The Roles of Animal Selection and Extrapolation (Roloff MV, ed). London:Taylor and Francis, 1987;187–193.
57. Leopold AS, Erwin M, Browning B. Phytoestrogens: adverse effects on reproduction in California quail. Science 191:98–100 (1976).
58. Adkins-Regan E, Mansukhani V, Seiwert C, Thompson R. Sexual differentiation of brain and behavior in the zebra finch: critical periods for effects of early estrogen treatment. J Neurobiol 25:865–877 (1994).
59. Gilbertson M, Kubiak T, Ludwig J, Fox G. Great Lakes embryo mortality, edema, and deformities syndrome (GLEMEDS) in colonial fish-eating birds: similarity to chick-edema disease. J Toxicol Environ Health 33:455–520 (1991).
60. Hart LE, Cheng KM, Whitehead PE, Shah RM, Lewis RJ, Ruschkowski SR, Blair RW, Bennett DC, Bandiera SM, Norstrom RJ, Bellward GD. Dioxin contamination and growth and development in great blue heron embryos. J Toxicol Environ Health 32:331–344 (1991).
61. Ohlendorf HM, Hoffman DJ, Saiki MK, Aldrich TW. Embryonic mortality and abnormalities of aquatic birds: apparent impacts of selenium from irrigation drainwater. Sci Total Environ 52:49–63 (1986).
62. Henny CJ, Herron GB. DDE, selenium, mercury, and white-faced ibis reproduction at Carson Lake Nevada. J Wildl Manage 53:1032–1045 (1989).
63. Hoffman DJ. Embryotoxicity and teratogenicity of environmental contaminants to bird eggs. Rev Environ Contam Toxicol 115:39–89 (1990).
64. Couillard CM. Bioassays for the toxicity of petroleum oils to birds. Ph.D. thesis, University of Saskatchewan, Saskatoon, SK, Canada, 1989.
65. Couillard CM, Leighton FA. Comparative pathology of Prudhoe Bay crude oil and inert shell sealants in chicken embryos. Fundam Appl Toxicol 13:165–173 (1989).
66. Couillard CM, Leighton FA. The toxicopathology of Prudhoe Bay crude oil in chicken embryos. Fundam Appl Toxicol 14:30–39 (1990).
67. Holmes WN. Some common pollutants and their effects on steroid hormone-regulated mechanisms. Aspects of avian endocrinology: practical and theoretical implications. Graduate Studies, Lubbock, TX:Texas Technical University, 26:365–370 (1982).
68. Fry DM, Addiego LA. Effects of oil exposure and stress on seabird endocrine systems. Proc Int Assoc Aquat Anim Med 19:60–68 (1988).

69. Trivelpiece W, Butler RG, Miller DS, Peakall DB. Reduced growth and survival of chicks of oil-dosed adult Leach's storm-petrels. Condor 86:81–82 (1981).
70. Ainley DG, Grau CR, Roudybush TE, Morrell SH, Utts JM. Petroleum ingestion reduces reproduction in Cassin's auklets. Mar Pollut Bull 12:314–317 (1981).
71. Albers PH. Effects of oil on avian reproduction: a review and discussion. In: The Effects of Oil on Birds: Physiological Research, Clinical Applications & Rehabilitation. Wilmington, DE:TriState Bird Rescue and Research, 1983;78–97.
72. Albers PH. Oil spills and the environment: a review of chemical fate and biological effects of petroleum. In: The Effects of Oil on Wildlife: Research, Rehabilitation, and General Concerns. Proceedings from The Oil Symposium, 16–18 October 1990, Herndon, Virginia. Hanover, PA:The Sheridan Press, 1991;1–12.
73. Leighton FA, Peakall DB, Butler RG. Heinz-body hemolytic anemia from the ingestion of crude oil: a primary toxic effect of marine birds. Science 20:871–873 (1983).
74. Fry DM, Lowenstine LJ. Pathology of common murres and Cassin's auklets exposed to oil. Arch Environ Contam Toxicol 14:725–737 (1985).
75. Fry DM, Addiego LA. Hemolytic anemia complicates the cleaning of oiled seabirds. Wildlife J 10:1–8 (1987).
76. Peakall DB, Norstrom RJ, Jeffrey DA, Leighton FA. Induction of hepatic mixed function oxidases in the herring gull (*Larus argentatus*) by Prudhoe Bay crude oil and its fractions. Comp Biochem Physiol 94C:461–463 (1989).
77. Fry DM, Swenson J, Addiego LA, Grau CR, Kang A. Reduced reproduction of wedge-tailed shearwaters exposed to single dose of 2 ml of weathered Santa Barbara crude oil. Arch Environ Contam Toxicol 15:453 –463 (1986).
78. Kolaja GJ, Hinton DE. DDT-induced reduction in eggshell thickness, weight, and calcium is accompanied by calcium ATPase inhibition. In: Animals as Monitors of Environmental Pollutants Symposium (Nielsen SW, Migaki G, Scarpelli DG, eds). Washington:National Academy of Sciences, 1979;309–318.
79. Lundblom CD. Effect of *p-p'*-DDE administered *in vivo* and *in vitro* on Ca^{2+} binding and $Ca^{2+}Mg^{2+}$–ATPase activity in eggshell gland mucosa of ducks. Acta Pharmacol Toxicol 50:121–129 (1982).
80. Schwarzbach SE, Fry DM, Rosson BE, Bird DM. Metabolism and storage of *p,p'*-dicofol in American kestrels (*Falco sparverius*) with comparisons to ring-neck doves (*Streptopelia risoria*). Arch Environ Contam Toxicol 20:206–210 (1990).
81. Gilman AP, Hallett DJ, Fox GA, Allan LJ, Learning WJ, Peakall DB. Effects of injected organochlorines on naturally incubated herring gulls eggs. J Wildl Manage 42:484–493 (1978).
82. McArthur MLB, Fox GA, Peakall DB, Philogene BJR. Ecological significance of behavioral and hormonal abnormalities in breeding ring doves fed on organochlorine chemical mixture. Arch Environ Contam Toxicol 12:343–353 (1983).
83. Fox GA, Tom D. Organochlorine pollutants, nest defense behavior and reproductive success in merlins. Condor 82:81–84 (1980).
84. Bennett RS, Williams BA, Schmedding DW, Bennett JK. Effects of dietary exposure to methyl parathion on egg laying and incubation in mallards. Environ Toxicol Chem 10:501–507 (1991).
85. White DH, Mitchell CA, Hill EF. Parathion alters incubation behavior of laughing gulls. Bull Environ Contam Toxicol 31:93–97 (1983).